

~~[casein hydrolysate and NH_4^+ and/or NO_3^-] a nitrogen source to form embryogenic callus;~~

~~(c) culturing said embryogenic callus on developmental medium containing an osmotic pressure increasing agent and cytokinin;~~

~~(d) culturing said embryogenic callus on maturation medium; and~~

~~(e) recovering poinsettia plants from said embryos.~~

~~Sub G1 (Three Times Amended) A method for producing transgenic poinsettia plants, comprising:~~

~~(a) incubating poinsettia plant tissue explants that produce reddish epidermal callus on auxin- and cytokinin-containing callus induction medium;~~

~~(b) culturing reddish epidermal callus on embryo induction medium comprising [casein hydrolysate and NH_4^+ and/or NO_3^-] a nitrogen source to form embryogenic callus;~~

~~(c)~~

~~(i) introducing an expression vector into said incubating embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or~~

~~(ii) introducing two expression vectors into said incubating embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene;~~

~~wherein the vector or vectors are introduced into the incubating embryogenic callus by co-incubating the callus with *Agrobacterium tumefaciens* containing the vector or vectors, by microprojectile-mediated delivery of the vector into the callus, or by electroporation;~~

- F2 concluded
- (d) culturing said transformed embryogenic callus on selection medium;
 - (e) culturing said transformed embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent;
 - (f) culturing said transgenic embryos on maturation medium; and
 - (g) recovering transgenic plants from said transgenic embryos.
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18. (Twice Amended) The method of claim 17, wherein said second foreign gene disrupts the function of said virus, and wherein said virus-disrupting gene is selected from the group consisting of genes encoding viral coat protein, 2'-5' oligonucleotide synthetase, viral genome antisense RNA, and pokeweed antiviral protein.

Sub G2 39. (Three Times Amended) A method for producing transgenic poinsettia plants, comprising:

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- (a) incubating poinsettia plant tissue explants that produce reddish epidermal callus in auxin- and cytokinin-containing callus induction medium;
 - (b) culturing embryogenic callus produced on said callus induction medium in liquid $[\text{NH}_4^+$ and/or NO_3^- containing] embryo induction medium comprising a nitrogen source;
 - (c) filtering the culture and culturing the filtrate in fresh liquid embryo induction medium;
 - (d) filtering the culture and culturing the filtrate on solid embryo induction medium;
 - (e) culturing embryos produced on said embryo [development] induction medium on maturation medium;
 - (f) culturing said embryos on callus induction medium;
 - (g) culturing epidermal callus produced on said callus induction medium on embryo induction medium to form embryogenic callus;

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(h)

(i) introducing an expression vector into said embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or

(ii) introducing two expression vectors into said embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene;

wherein the vector or vectors are introduced into the incubating embryogenic callus by co-incubating the callus with *Agrobacterium tumefaciens* containing the vector or vectors, by microprojectile-mediated delivery of the vector into the callus, or by electroporation;

(i) culturing said transformed embryogenic callus on selection medium;

(j) culturing said transformed embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent;

(k) culturing said transformed embryos on maturation medium; and

(l) recovering transgenic plants from said transgenic embryos.

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52. (Twice Amended) The method of claim 51, wherein said second foreign gene disrupts the function of said virus, and wherein said virus-disrupting gene is selected from the group consisting of genes encoding viral coat protein, 2'-5' oligonucleotide synthetase, viral genome antisense RNA, and pokeweed antiviral protein.

53. (Twice Amended) The method of claim [51] 39, wherein said second foreign gene confers resistance to an insect, and wherein said insect resistance gene encodes a protein selected

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from the group consisting of tryptophan decarboxylase, lectin, and *Bacillus thuringiensis* toxin.

76. (Twice Amended) The [method] transgenic poinsettia plant of claim 73, wherein the expression of said [second] foreign gene confers resistance to disease caused by an organism selected from the group consisting of virus, bacterium, and fungus.

77. (Twice Amended) The [method] transgenic poinsettia plant of claim 76, wherein said [second] foreign gene disrupts the function of said virus, and wherein said virus-disrupting gene is selected from the group consisting of genes encoding viral coat protein, 2'-5' oligonucleotide synthetase, viral genome antisense RNA, and pokeweed antiviral protein.

78. (Twice Amended) The [method] transgenic poinsettia plant of claim [70] 73, wherein said [second] foreign gene confers resistance to an insect, and wherein said insect resistance gene encodes a protein selected from the group consisting of tryptophan decarboxylase, lectin, and *Bacillus thuringiensis* toxin.

101. (Twice Amended) A method for *in vitro* regeneration of poinsettia plants comprising:

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- (a) incubating poinsettia plant tissue explants that produce epidermal callus on auxin- and cytokinin-containing callus induction medium;
 - (b) subculturing reddish epidermal callus to $[\text{NH}_4^+ \text{ and/or } \text{NO}_3^- \text{ containing}]$ embryo induction medium comprising a nitrogen source to form embryogenic callus;
 - (c) culturing said embryogenic callus on developmental medium containing an osmotic pressure increasing agent and cytokinin;
 - (d) culturing said embryogenic callus on maturation medium; and
 - (e) recovering poinsettia plants from said embryos.
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102. (Twice Amended) A method for producing transgenic poinsettia plants comprising the steps of:

- (a) incubating poinsettia plant tissue explants that produce epidermal callus on auxin- and cytokinin-containing callus induction medium;
- (b) culturing reddish epidermal callus to embryo induction medium comprising a nitrogen source to form embryogenic callus containing embryos;

(c) (i) introducing an expression vector into said incubating embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or

(ii) introducing two expression vectors into said incubating embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene;

(d) culturing said transformed embryogenic callus on selection medium;

(e) culturing said embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent;

(f) culturing said transgenic embryos on maturation medium; and

(f) recovering transgenic plants from said transgenic embryos.

103. (Amended) A method for producing transgenic poinsettia plants comprising the steps of:

(a) incubating poinsettia plant tissue explants that produce epidermal callus on auxin- and cytokinin-containing callus induction medium;

(b) culturing embryogenic callus produced on said callus induction medium in liquid

- embryo induction medium comprising a nitrogen source;
- (c) filtering the culture and culturing the filtrate in fresh liquid embryo induction medium;
- (d) filtering the culture and culturing the filtrate on solid embryo induction medium;
- (e) culturing embryos produced on said embryo [development] induction medium on maturation medium;
- (f) culturing said embryos on callus induction medium;
- (g) culturing epidermal callus produced on said callus induction medium on embryo induction medium to form embryogenic callus containing embryos;
- (h)
- (i) introducing an expression vector into said incubating embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or
- (ii) introducing two expression vectors into said incubating embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene;
- (i) culturing said transformed embryogenic callus on selection medium;
- (j) culturing said transformed embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent;
- (k) culturing said transformed embryos on maturation medium; and
- recovering transgenic plants from said transgenic embryos.

F9 111. (Amended) The method of claim [49] 39, wherein the expression of said second foreign gene confers resistance to an insect.

Please add the following new claims:

113. The method according to claim 1, wherein the nitrogen source comprises NH_4^+ and/or NO_3^- .

114. The method according to claim 6, wherein the nitrogen source comprises NH_4^+ and/or NO_3^- .

F10 115. The method according to claim 39, wherein the nitrogen source comprises NH_4^+ and/or NO_3^- .

116. The method according to claim 101, wherein the nitrogen source comprises NH_4^+ and/or NO_3^- .

117. The method according to claim 102, wherein the nitrogen source comprises NH_4^+ and/or NO_3^- .

118. The method according to claim 103, wherein the nitrogen source comprises casein hydrolysate and NH_4^+ and/or NO_3^- .

REMARKS

Amendments

Claims 18, 39, 52, 53, 76-78, 101, and 111 have been amended for clarity as suggested in the Final Office Action. Claims 1 and 101 have been amended to recite that the developmental medium comprises cytokinin. Support for this amendment can be found in the specification at, *inter alia*, page 18, first paragraph and page 26, table 4.

Claims 102 and 103 have been amended to recite that the callus induction medium comprises auxin and cytokinin and that the developmental medium comprises an osmotic